Sequence Revision History

Revision history for "AE001641"			
GI	**Version**	**Update Date**	**Status**
4376855	1	Dec 1 2000 9:38	Live
4376855	1	Oct 30 2000 12:10	Dead
4376855	1	Mar 8 1999 17:32	Dead

Accession AE001641 was first seen at NCBI on Mar 8 1999 17:32

BLAST

Reference sequence project

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Protocol: Making antibodies to synthetic peptides

You'll need to couple the synthetic peptide hapten to a protein carrier, with a bifunctional reagent.

For antibody development, we don't bother to use highly purified peptide. Crude (unpurified) peptide or partially purified peptide does just fine; after all, the impurities in unpurified peptides are principally incomplete, partial versions of the desired peptide. Since you need to use quite a bit of peptide to get good coupling and antibody production (10s of mg), it's probably most economical to use unpurified synthetic peptides.

The most useful coupling protocols are in: RF Doolittle. Of Urfs and Orfs. A Primer on How to Analyze Dervied Amino Acid Sequences. University Science Books, 1987.

Good carriers include bovine serum albumin, or keyhole limpet hemocyanin (our favorite, from Calbiochem #374817).

Good coupling methods are glutaraldehyde (amino to amino), MBS (=m-Maleimidobenzoic acid-N-hydroxysuccinimide; amino to sulfhydryl), BDB (bisdiazobenzidine; couples tyr to tyr), EDAC (carbodiimide; couples amino to carboxyl).

We often add an extra ("adventitious") tyr to the N- or C-terminus of the synthetic peptide, for BDB coupling. We've done this more often than not. Try to put the extra tyr at the least "interesting" end of the peptide, since it will be attached (buried) to the carier, and hence you'll tend to get antibody to the most interesting end of the peptide (away from the carrier).

It's useful to have a very low specific activity (say, 10,000 cpm/mg peptide) 14C label in a gly or ala residue in the peptide. You can then follow coupling efficiency of the peptide into the carrier.

We typically immunize rabbits by the Vaitukaitis protocol, with multiple intradermal immunizations at once. We shave the upper back of the rabbit prior to immunization. We tend to use 1 mg of peptide per immunization (3-5 intradermal sites at each immunization), and re-immunize at 2-6 week intervals. Intradermal (intracutaneous) means: raise a wheal (inject into the skin, just a mm or so beneeath the surface). After the initial immunization and 2-3 boosts, we bleed 3-4 weeks later, and test the antibodies, by western blot (immunoblot) or immunoprecipitation of [125-I]-labeled antigen (either the peptide, or the whole molecule from which the peptide derives).

For the initial immunization, we mix the immunogen (hapten-carrier conjugate) with an equal volume of complete Freund's adjuvant. For boosts, we use incomplete Freund's adjuvant. Mixing the oily adjuvant with the aqueous conjugate is best done with a mixing needle and two attached syringes. Mixing results in a suspension.

By this kind of protocol, we ALWAYS obtain synthetic peptide antibodies which recognize the antigen (intact full length molecule, from which the peptide was derived), especially on immunoblot. Sometimes the epitope is weak, and the antobodies are of low titer (e.g., 1:100 for immunoblot). But we always get a usable antibody.

Citations where we've used these procedures:

Barbosa JA, Gill BM, Takiyyuddin MA, O'Connor DT: Chromogranin A: posttranslational modifications in secretory granules. Endocrinology 128:174-190, 1991. Here, we used antibodies to chromogranin A synthetic peptides to examine route of proteolysis (post-translational processing) of the molecule.

Gill BM, Barbosa JA, Dinh TQ, Garrod S, O'Connor DT: Chromogranin B: isolation from pheochromocytoma, N-terminal sequence, tissue distribution and secretory vesicle processing. Regul Peptides 33:223-35, 1991. Here, we used antibodies to chromogranin B synthetic peptides to examine route of proteolysis of the molecule.

Gill BM, Barbosa JA, Hogue-Angeletti R, Varki N, O'Connor DT: Chromogranin A epitopes: clues from synthetic peptides and peptide mapping. Neuropeptides 21:105-18, 1992. Here, we used antibodies to chromogranin A synthetic peptides to immunoprecipitate the molecule.

Takiyyuddin MA, DeNicola L, Gabbai FB, Dinh TQ, Kennedy B, Ziegler MG, Sabban EL, Parmer RJ, O'Connor DT: Catecholamine secretory vesicles. Augmented chromogranins and amines in secondary hypertension. Hypertension 21:674-9, 1993. Here we developed an RIA for chromogranin A, based on an N-terminal synthetic peptide.